Quick-Start Protocol August 2018

RNeasy® PowerMicrobiome® Kit

Lyophilized DNase I should be stored at 2–8°C upon arrival. All other reagents and components of the RNeasy PowerMicrobiome Kit should be stored at room temperature (15–30°C). DNase I is sensitive to physical denaturation; do not vortex resuspended DNase I.

Further information

- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- Solution PM1 must be warmed at 55°C for 5–10 minutes prior to use.
- Shake to mix Solution PM5 before use.
- Prepare Solution PM1 by adding 10 µl β-mercaptoethanol (β-ME) for every 990 µl Solution PM1 (a total of 1 ml for each prep).
- Prepare DNase I stock enzyme by adding 550 µl RNase-Free Water to the DNase I (RNase-free) lyophilized powder and mixing gently. Aliquot the DNase I stock enzyme in 50 µl portions and store at –30°C to –15°C for long-term storage (but do not freeze—thaw more than 3 times). To prepare DNase I Solution, thaw and combine 5 µl DNase I stock enzyme with 45 µl DNase Digestion Solution per prep.

1. Place 0.25 g of stool or biosolid sample into a PowerBead Bead Tube, Glass 0.1 mm. **Note:** If phenol-based lysis is desired, add 100 µl phenol–chloroform–isoamyl alcohol (25:24:1, pH 6.5–8.0) to the PowerBead Tube before adding the sample.
2. Add 650 µl Solution PM1—βME to the PowerBead Tube. Alternatively, you may add 650 µl PM1 and 6.5 µl βME to the PowerBead Tube.
3. Secure the PowerBead Tube horizontally to a Vortex Adapter (cat. no. 13000-V1-24). Orient tube caps to point toward the center of the Vortex Adapter.
4. Vortex at maximum speed for 10 min. Centrifuge at 13,000 x *g* for 1 min at room temperature. Transfer the supernatant to a clean 2 ml Collection Tube (provided). **Note:** If you added phenol–chloroform–isoamyl alcohol, remove the upper aqueous layer and transfer to a clean 2 ml Collection Tube (provided).
5. Add 150 µl Solution IRS and vortex briefly to mix. Incubate at 2–8°C for 5 min.

6. Centrifuge at 13,000 x *g* for 1 min.
7. Avoiding the pellet, transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: Do not transfer more than 650 µl at this step.
8. Add 650 µl each of Solution PM3 and Solution PM4. Vortex briefly to mix.
   Note: To prevent small RNAs (5S RNAs, tRNAs and degraded RNAs) from co-purifying with mRNA and rRNA, use 650 µl 70% ethanol instead of Solution PM4. To purify small RNAs, such as microRNAs and siRNAs, transfer the lysate to a larger tube to accommodate a higher volume (2.6 ml) and add an additional 650 µl 100% ethanol (user supplied) to the lysate.
9. Load 650 µl supernatant into an MB RNA Spin Column and centrifuge at 13,000 x *g* for 1 min. Discard the flow-through, and repeat until all the supernatant has been processed through the Spin Column.
10. Shake to mix Solution PM5. Add 650 µl Solution PM5 to the MB RNA Spin Column and centrifuge at 13,000 x *g* for 1 min.
    Note: Skip steps 11–13 if you want to isolate both RNA and DNA.
11. Discard flow-through and centrifuge at 13,000 x *g* for 1 min to remove residual wash.
12. Place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided). To the center of the Spin Column, add 50 µl DNase I Solution (prepared by mixing 45 µl DNase Digestion Solution and 5 µl DNase I stock enzyme; see "Notes before starting").
13. Incubate at room temperature for 15 min. Add 400 µl Solution PM7 and centrifuge at 13,000 x *g* for 1 min.
14. Discard flow-through. Add 650 µl Solution PM5. Centrifuge at 13,000 x *g* for 1 min.
15. Discard flow-through. Add 650 µl Solution PM4. Centrifuge at 13,000 x *g* for 1 min.
16. Discard-flow through. Centrifuge at 13,000 x *g* for 2 min.
17. Place the MB RNA Spin Column into a clean 2 ml Collection Tube (provided).
18. Add 100 µl RNase-Free Water (provided) to the center of the white filter membrane. Incubate at room temperature for at least 1 min.
    Note: Eluting with 100 µl RNase-Free Water will maximize RNA yield. For more concentrated RNA, elute using a **minimum** of 50 µl RNase-Free Water.
19. Centrifuge at 13,000 x *g* for 1 min. Discard the MB RNA Spin Column. The RNA is now ready for any downstream application.